University of Warsaw
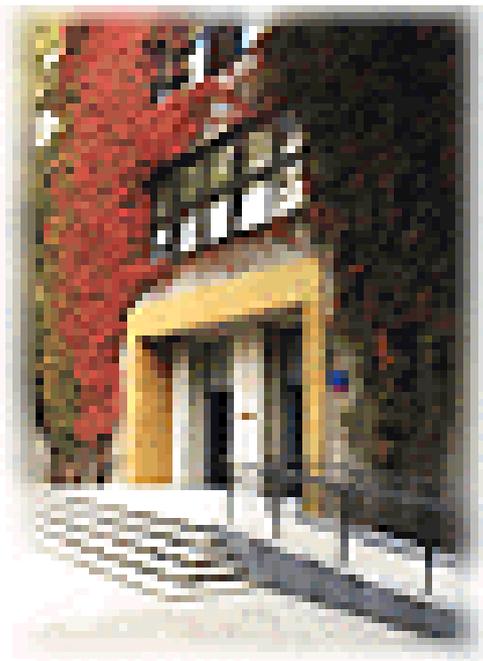Faculty of Chemistry
Biological and Chemical Reserach Centre

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Biologiczno-Chemicznych
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CRMs and benefits of their use for the laboratory

Ewa Bulska

Project from NCBIR: „Production and certification of reference materials for environmental purposes
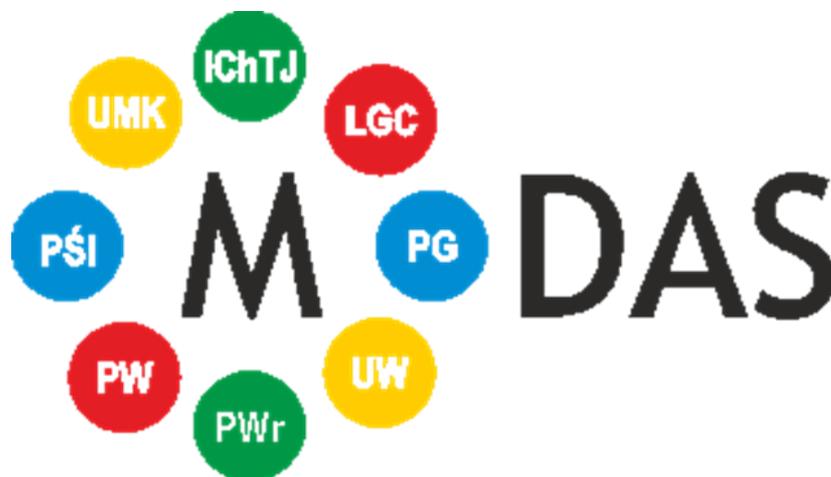
ISO/IEC 17025 (QA) requirements

- ✓ Test and calibration methods and method validation (5.4)
- ✓ Equipment (5.5)
- ✓ Measurement traceability (5.6)
- ✓ Quality of test and calibration results (5.9)

→ Requirements for reagents, calibrants and measurement standards for validation of methods
→ Proper use of (certified) Reference Materials

- ✓ Suitable laboratory environment
- ✓ Suitable equipment
- ✓ Educated, trained and skilled staff
- ✓ Training procedures and records

→ Procedures for checking and reporting results
→ Proper storage and handling of samples
→ Participation in proficiency tests

ISO/IEC 17025

5.4.5.2 The laboratory shall validate non-standard methods, laboratory-designed/developed methods, standard methods used outside their intended scope, and amplifications and modifications of standard methods to confirm that the methods are fit for the intended use. The validation shall be as extensive as is necessary to meet the needs of the given application or field of application. The laboratory shall record the results obtained, the procedure used for the validation, and a statement as to whether the method is fit for the intended use.

NOTE 1 Validation may include procedures for sampling, handling and transportation.

NOTE 2 The techniques used for the determination of the performance of a method should be one of, or a combination of, the following:
  ☐ calibration using reference standards or reference materials;
  ☐ comparison of results achieved with other methods;
  ☐ interlaboratory comparisons;
  ☐ systematic assessment of the factors influencing the result;
  ☐ assessment of the uncertainty of the results based on scientific understanding of the theoretical principles of the method and practical experience

ISO/IEC 17025

5.4.5.2 The laboratory shall validate …..

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 ☐ assessment of the uncertainty of the results based on scientific understanding of the theoretical principles of the method and practical experience

ISO/IEC 17025

5.6.2.1.2 There are certain calibrations that currently cannot be strictly made in SI units. In these cases calibration shall provide confidence in measurements by **establishing traceability** to appropriate measurement standards such as:

☐ **the use of certified reference materials provided by a competent supplier to give a reliable physical or chemical characterization of a material;**

☐ the use of specified methods and/or consensus standards that are clearly described and agreed by all parties concerned.

ISO/IEC 17025

5.9.1 The laboratory shall have quality control procedures for **monitoring the validity of tests and calibrations undertaken**. The resulting data shall be recorded in such a way that trends are detectable and, where practicable, statistical techniques shall be applied to the reviewing of the results.

This monitoring shall be planned and reviewed and may include, but not be limited to, the following:

a) regular use of certified reference materials and/or internal quality control using secondary reference materials;

Metrological comparability of results (**traceability**)

C_0

C_{RM1} C_{RM2}

C_1 C_2
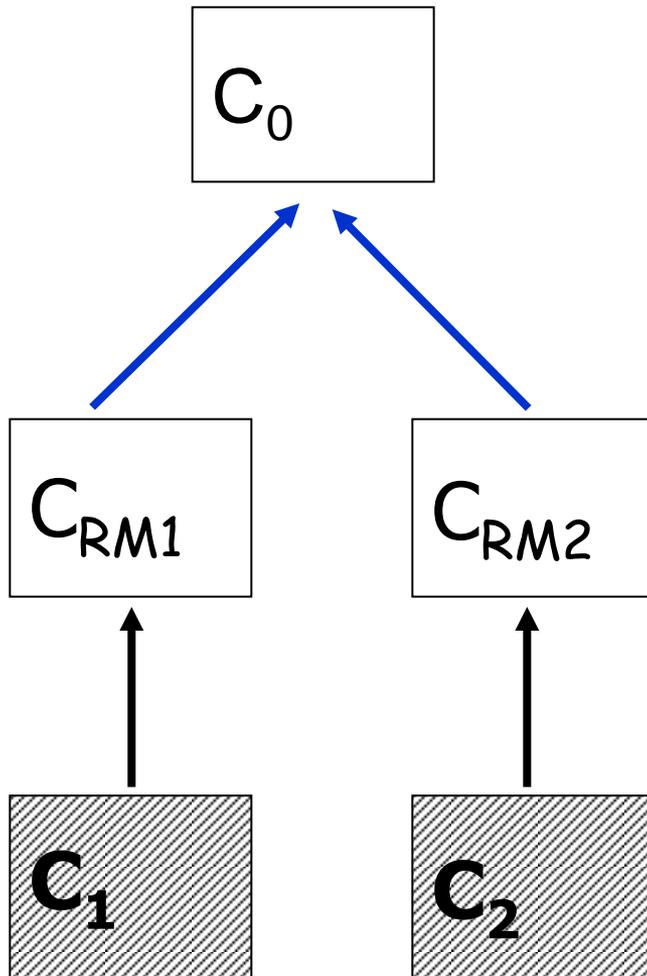
Yes, through common reference

VIM 3 : Definition

Metrological traceability

is a property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations each contributing to the measurement uncertainty.

[VIM3, 2.41]

VIM 3 : Definition

Reference Material (RM) is a material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in a **measurement** process or in examination of **nominal properties**

(VIM 3, ISO/IEC Guide 99:2007 (E/F)

VIM 3 : Definition

Certified Reference Material (CRM) is a reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with **associated uncertainties and traceabilities,** using valid procedures

(VIM 3, ISO/IEC Guide 99:2007 (E/F)

VIM 3 : Definition

Note 2. RM with or without assigned quantity values can be used for **measurement precision** control whereas only RM with assigned quantity values can be used for **calibration or measurement trueness** control.

(VIM 3, ISO/IEC Guide 99:2007 (E/F)

Selection of CRMs

- Type of matrix
- Concentration range
- Homogenity
- Stability
- Uncertainty (contribution to uncertainty budget)
- Value assignment procedures (measurement and statistical)
- Value: certified / recomended
- Producer
- Cost

The use of CRMs

- The intended use
- Sample preparation (instruction given by supliers)
- prescribed minimum test portion
- storage temperature (-20, +4, +18 oC)
- humidity/moisture uptake (e.g. biological activity)
- results recalculated (e.g. dry mass, if necessary)
- expiry date

FOSFATAZA ZASADOWA ALKALINE PHOSPHATASE (ALP)	IFCC, bufor AMP z p-nitrofenylofosforanem	197	U/l (37°C)	155 - 238
	p-Nitrophenylphosphate, AMP buffer (IFCC)	126	U/l (25°C)	99 - 152
	DGKC, bufor DEA z p-nitrofenylofosforanem	278	U/l (37°C)	219 - 336
	p-Nitrophenylphosphate, DEA buffer (DGKC)	178	U/l (25°C)	140 - 215
	Sucha chemia / Dry chemistry	187	U/l	147 - 226

FOSFATAZA ZASADOWA ALKALINE PHOSPHATASE (ALP)	IFCC, bufor AMP z p-nitrofenylofosforanem	349	U/l (37°C)	276 – 422
	p-Nitrophenylphosphate, AMP buffer (IFCC)	223	U/l (25°C)	176 – 270
	DGKC, bufor DEA z p-nitrofenylofosforanem	454	U/l (37°C)	359 – 550
	p-Nitrophenylphosphate, DEA buffer (DGKC)	290	U/l (25°C)	229 – 351
	Sucha chemia / Dry chemistry	304	U/l	240 – 368

Procedure dependent reference values

The method is specified!

LGC6139(RM)   Metals in River clay sediment
The extractable metal content refers to metals soluble in hot Aqua Regia using method ISO11466 (1995). The total metals were determined using at least two of the following procedures: chemical dissolution techniques, X-ray fluorescence and NAA.

Ref. values:
Total Metals – Al 5.7%, Ba 584 mg kg^{-1}, Ca 4.2%, Cr 126 mg kg^{-1}, Cu 96 mg kg^{-1}, Fe 3.2%, K 1.8%, Mg 1.2%, Mn 0.11%, Na 0.58%, Ni 44 mg kg^{-1}, Pb 176 mg kg^{-1}, Rb 108 mg kg^{-1}, Si 27.4%, Sr 154 mg kg^{-1}, Ti 0.36%, Zn 530 mg kg^{-1}
Extractable Metals – Cd 2.3 mg kg^{-1}, Cr 80 mg kg^{-1}, Cu 92 mg kg^{-1}, Hg 1.2 mg kg^{-1}, Pb 160 mg kg^{-1}, Ni 38 mg kg^{-1},, Se 0.59 mg kg^{-1}, Zn 513 mg kg^{-1}

Drying

- Sometimes it is necessary to include correction for humidity. Example:

The sample must be used as it is in the bottle, but the reference values apply to the dry mass!

The humidity correction factor is derived from a separate portion of the CRM!

CRM BCR 700 EXTRACTABLE TRACE ELEMENTS IN ORGANIC-RICH SOIL

... The sample must be used as it is from the bottle. The correction to dry mass should be made on a separate portion of 1 g which should be dried in an oven at (105 ± 2) °C for 2-3 h until constant mass is attained. ...

5.1 Method Validation and Measurement Uncertainty Estimation of bias (the difference between the measured value and the true value) is one of the most difficult elements of method validation, but appropriate RMs can provide valuable information, within the limits of the uncertainty of the RMs certified value(s) and the uncertainty of the method being validated. Although traceable certified values are highly desirable, the estimation of bias differences between two or more methods can be established by use of less rigorously certified RMs. Clearly the RMs must be within the scope of the method in terms of matrix type, analyte concentration etc. and ideally a number of RMs covering the full range of the method should be tested. Where minor modifications to a well-established method are being evaluated then less rigorous bias studies can be employed. Replicate measurement of the RM, covering the full range of variables permitted by the method being validated can be used to estimate the uncertainty associated with any bias, which should normally be corrected for (15). The uncertainty associated with an RM should be no greater than one third of that of the sample measurement.

5.2 Verification of the Correct Use of a Method The successful application of a valid method depends on its correct use, both with regard to operator skill and suitability of equipment, reagents and standards. RMs can be used for training, for checking infrequently used methods and for trouble shooting when unexpected results are obtained.

5.3 Calibration Normally a pure substance RM is used for calibration of the measurement stage of a method. Other components of the test method, such as sample digestion, separation and derivatisation are, of course, not covered and loss of analyte, contamination and interferences and their associated uncertainties must be addressed as part of the validation of the method. The uncertainty associated with RM purity will contribute to the total uncertainty of the measurement. For example, an RM certified as 99.9% pure, with an expanded uncertainty U (k=2) of 0.1% will contribute an uncertainty component of 0.1% to the overall measurement uncertainty budget. In the case of trace analysis, this level of uncertainty will rarely be important but for assay work, it can be expected to be significant. Some other methods, such as XRF analysis, use matrix RMs for calibration of the complete analytical process. In addition to a close matrix match, the analyte form must be the same in the samples and RMs, and the analytical concentrations of the RMs must span that of the samples (12). ISO Guide 32 and reference 7 provide additional useful information.
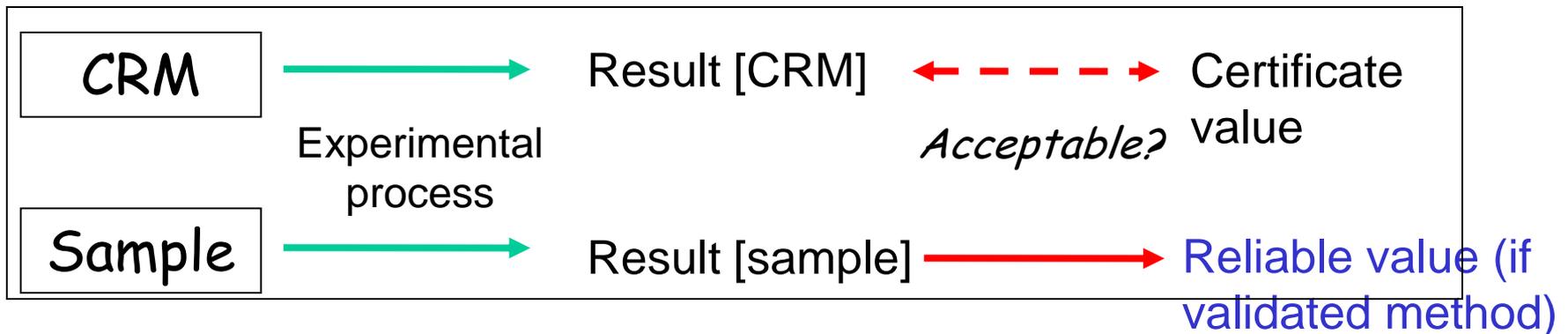
5.4 Quality Control and Quality Assurance (QC&QA) RMs should be characterised with respect to homogeneity, stability, and the certified property value(s). For in-house QC, however, the latter requirement can be relaxed, but adequate homogeneity and stability are essential. Similar requirements apply to samples used to establish how well or badly measurements made in different laboratories agree. In the case of proficiency testing, homogeneity is essential and sample stability within the timescale of the exercise must be assessed and controlled. Although desirable, the cost of certifying the property values of proficiency testing samples often prohibits this being done and consensus mean values are often used instead. As a consequence, there often remains some doubt concerning the reliability of assigned values used in proficiency testing schemes. This is because, although the consensus mean of a set of data has value, 'the majority' is not necessarily correct and as a consequence the values carry some undisclosed element of uncertainty. The interpretation of proficiency testing data thus needs to be carried out with caution.

(C)RMs can be used for:
Calibration as part of a measurement procedure
pure substances
matrix CRMs (e.g. analysis of steel by XRF)
Validation of measurement procedure
Quality control and quality assurance
Comparison with in-house materials

"matrix" CRMs available
2.1 matrix "matching"
(sediment sample; sediment CRM; similar
concentration range)

CRM	→ Experimental process →	Result [CRM]	← - - - → Acceptable?	Certificate value
Sample	→	Result [sample]	→	Reliable value (if validated method)

Similar matrix AND similar analyte concentration

- serum sample v.s. serum CRM
- steel v.s. steel CRM
- natural water v.s. water CRM

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